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D1 receptors play a major role in the dopamine modulation of mouse ileum contractility

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ABSTRACT

Since the role of dopamine in the bowel motility is far from being clear, our aim was to analyse pharmacologically the effects of dopamine on mouse ileum contractility. Contractile activity of mouse ileum was examined *in vitro* as changes in isometric tension. Dopamine caused a concentration-dependent reduction of the spontaneous contraction amplitude of ileal muscle up to their complete disappearance. SCH-23390, D1 receptor antagonist, which *per se* increased basal tone and amplitude of spontaneous contractions, antagonized the responses to dopamine, whilst sulpiride or domperidone, D2 receptor antagonists, were without effects. The application of both D1 and D2 antagonists had additive effects. SKF-38393, D1 receptor agonist, mimicked dopamine-induced effects. Dopamine responses were insensitive to tetrodotoxin, atropine, nitric oxide synthase inhibitor or adenosine receptor antagonists, but they were reduced by adenylyl cyclase inhibition or apamin. Dopamine at a concentration which did not cause a significant reduction of phasic contractions inhibited the cholinergic contractions in response to field stimulation. SCH-23390 *per se* induced an increase of the neural cholinergic contraction and antagonized the dopamine effects, whilst sulpiride or domperidone did not. The application of D1 and D2 antagonists had additive effects. In conclusion, mouse ileum is under basal inhibitory control by dopamine, through D1 receptor activation, linked to adenylyl cyclase and activation of apamin-sensitive potassium channels. An agonistic interaction of the dopamine receptor subtypes in the regulation intestinal contractility has being also highlighted. This study would provide new insight on the pharmacology of the modulation of the gastrointestinal contractility by dopamine.

1. Introduction

It is well known that catecholamines modulate gastrointestinal (GI) motility. Sympathetic nerves through norepinephrine inhibit acetylcholine (ACh) release from motor neurons (via α_2-adrenoceptors) [1] and relax smooth muscle [2]. The gut, however, also contains dopamine, which only recently has been confirmed as an intrinsic neurotransmitter of the enteric nervous system (ENS) [3,4]. In fact, enteric dopaminergic neurons, which express tyrosine hydroxylase and the dopamine transporter (DAT) but lack dopamine β-hydroxylase, enzyme that converts dopamine to norepinephrine, have been identified in mouse, guinea pig [4], and human [3]. In addition, non-neural cells belonging to a local dopaminergic autocrine and paracrine system are identifiable throughout the length of the gastrointestinal tract [5–7].

There are five subtypes of dopamine receptors, which can be grouped into two families: D1-like family, including D1 and D5 receptors, and D2-like family, including D2, D3, and D4 receptors [8,9]. In mouse, all five classes of dopamine receptors have been identified throughout the digestive tract [10,11]. In particular, transcripts encoding D1, D3, and D5 receptors are expressed both in nerve-containing layers of the gut and in the mucosa; transcripts encoding D2 receptors are restricted to neurons; whilst transcripts encoding D4 are confined to the mucosal layer. Thus, except D4 receptors each of the others potentially may mediate dopaminergic control of motility, being D2 receptors likely important mediators of neuronal responses to dopamine.

The function of enteric dopaminergic neurons in the regulation of GI motility is not clear and the mechanism of dopamine action and location of dopamine receptors are controversial. Dopamine relaxes the rat jejunum [12,13] and the dog colon [14]. In guinea pig stomach and rabbit ileum dopamine-mediated inhibition of GI

Abbreviations: ACh, acetylcholine; BK_{Ca}, large-conductance Ca^{2+}-activated K^+ channel; DDA, 2′,3′-dideoxyadenosine; DAT, dopamine transporter; DMPX, 3,7-dimethyl-1-propargylxanthine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; ENS, enteric nervous system; GI, gastrointestinal; IK_{Ca}, intermediate conductance Ca^{2+}-activated K^+ channel; L-NAME, N_ω-nitro-L-arginine methyl ester; MRS 1220, 9-chloro-2-(2-furanyl)-5-((phenylacetyl)amino)-[1,2,4]triazolo [1,5-c]quinazoline; SCH-23390, 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; SK_{Ca}, small conductance Ca^{2+}-activated K^+ channel; TTX, tetrodotoxin.

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motility occurs through modulation of the enteric nervous system, whereas in gastric tissue from opossum dopamine effects are mediated by dopamine receptors on smooth muscle cells [15]. It is generally accepted that in gut D1 receptors are mainly located postjunctionally, whereas D2 receptors are located pre- and postjunctionally. In mouse colon endogenously released dopamine inhibits both spontaneous phasic and electrically induced contraction amplitude and this inhibition is enhanced in DAT knock-out mice and restored to normal by the combined inhibition of D1 and D2 receptors [16]. Analysis of the propulsive gastrointestinal activity in transgenic mice lacking D2 receptors suggests that endogenous dopamine may act via axonal D2 receptors [12]. The activation of such receptors inhibits the release of ACh from enteric neurons thereby decreasing the strength of neurotransmission in prokinetic pathways. In fact, anti-dopaminergic drugs (i.e. domperidone and sulpiride) that act by blocking the D2 receptors are prokinetic and promote the motility of the gut. Lastly, conclusions regarding the role of dopamine in modulating GI tract motility have been confounded by the ability of dopamine agonists to activate adrenergic receptors [13,14,17].

Thus more research is required to improve our understanding of dopamine-mediated regulation of GI motility, also in consideration of a possible impact of dopaminergic dysfunction in human diseases. For example, the prototypical parkinsonian neurotoxin, MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), a selective dopamine neuron toxin in the enteric nervous system (ENS), causes a remarkable reduction of tyrosine hydroxylase positive neurons in the ENS of mice. Isometric recording of neural-mediated muscle contractions in isolated colon from MPTP-treated animals pointed out a relaxation defect associated with dopaminergic degeneration. Behaviorally, MPTP induced a transient increase in colon motility, but no changes in gastric emptying or small intestine transit [18].

In consideration that the role of dopamine in the bowel motility is far from being clear, the aim of this study was to analyse pharmacologically the effects of dopamine on mouse ileum contractility, and to characterize the subtype(s) of receptors involved and the related transduction mechanism. Small intestine was chosen because it is reported that in the murine ENS dopamine neurons are more numerous in the proximal than in the distal GI tract [4].

2. Materials and methods

2.1. Animals

All animal procedures were in conformity with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC). Experiments were performed on adult male mice (C57BL/10SnJ; 25.5 ± 0.5 g body weight; 15 weeks old), obtained from Charles River Laboratories (Calco-Lecco, Italy) and maintained in a light (12 h/12 h light) and temperature (23 °C) controlled environment with free access to food and water.

2.2. Recording of mechanical activity

Animals were sacrificed by cervical dislocation, the abdomen was immediately opened, and the ileum was removed and placed in Krebs solution consisting of (mM): NaCl 119; KCl 4.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.1. The contents of the excised segments were gently flushed out with Krebs solution. Segments, oriented along the longitudinal axis (20 mm in length), were suspended in a four channel organ bath containing 10 ml of oxygenated (95% O_2 and 5% CO_2) Krebs solution at 37 °C. The distal end of each segment was tied to an organ holder and the proximal end was secured with a silk thread to an isometric force transducer (FORT 10, Ugo Basile, Biological Research Apparatus, Comerio VA, Italy). Mechanical activity was digitized on an A/D converter, visualized, recorded and analysed on a personal computer using the PowerLab/400 system (Ugo Basile, Italy). Preparations were subjected to an initial tension of 200 mg and were allowed to equilibrate for at least 30 min. Rhythmic spontaneous contractions of varying amplitude developed in all preparations. In a series of experiments, ileal segments were electrically stimulated to evoke cholinergic contractions. Stimulation was provided via two parallel platinum electrodes and it was conducted at the 4 Hz as square-wave pulses of supramaximal voltage (0.5 ms) delivered by a Grass S88 electrical stimulator (Grass Instruments Co., Quincy, MA, USA) through a stimulus isolation unit (SIU5) using direct coupling. In all studies, the duration of the electrical stimulation was 10 s.

2.3. Experimental protocol

After the equilibration time, preparations were challenged with either 0.1 μM isoproterenol or with 10 μM carbachol for 2 min, until stable responses were obtained. Concentration–response curves for dopamine or D1 receptor agonist were constructed by non-cumulative addition of the drugs, applied for approximately 3 min at 20 min intervals. The antagonists were allowed to maintain contact with the tissue for at least 30 min before eventually repeating the curve of dopamine. Time control experiments showed that a second curve to the agonist was reproducible. Each preparation was tested with a single antagonist, except when otherwise stated. Concentrations of the drugs used were determined from literature. In order to evaluate the related mechanism of action, a submaximal dose of dopamine (30 μM) was tested in the presence of different antagonists/inhibitors, left in contact to the tissue for at least 20 min.

In a second set of experiments, due to the effects of the D1 receptor antagonist on the spontaneous activity, SCH-23390 (3–10 μM) was tested after pretreatment of 30 min with TTX, methysergide. Lastly, experiments of EFS were performed by stimulating the tissues with individual trains at 4 Hz delivered at 5 min intervals. Atropine abolished the EFS-induced contraction indicating its cholinergic nature. Subsequently, dopamine at the dose of 0.3 μM was added to the organ bath and the EFS was repeated after 2 min. When the effects of dopamine antagonists on EFS had to be tested, drugs were left in contact to the tissue for a period of 20–30 min.

2.4. Solution and drugs

The following drugs were used: apamin, atropine sulfate, carbachol (CCh), charybdotoxin dopamine, 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH-23390), 9-chloro-2-(2-furanyl)-5-((phenylacetyl)amino)-[1,2,4]triazolo[1,5-c]quinazoline (MRS 1220), 2′,3′-dideoxyadenosine (DDA), 3,7-dimethyl-1-propargylxanthine (DMPX), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), domperidone, N-1-(ethylpyrrolidin-2-ylmethyl)-2-methoxy-5-sulfamoyl benzamide, (±)-5-(aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl) methyl]-2-methoxybenzamide (sulpiride), Nω-nitro-L-arginine methyl ester (L-NAME), phentolamine hydrochloride, propanolol hydrochloride, (R)-(+)-SKF-38393 hydrochloride, tetrodotoxin (TTX), yohimbine hydrochloride (Sigma–Aldrich, Inc., St. Louis, USA). 1-(2-Ethylphenoxy)-3-[[(1S)-1,2,3,4-tetrahydro-1-naphthalenyl]amino]-(2S)-2-propanol hydrochloride (SR 59230A) was from Tocris-Bioscience (Bristol, UK) and methysergide hydrogen maleate was from Sandoz (Basle, Switzerland). Sulpiride and domperidone were dissolved in 0.1 M HCl; DMPX, phentolamine and propranolol were dissolved in ethanol; DDA, DPCPX, MRS1220